

Complete Listing of Claims (Clean Version)

1. (Withdrawn) A hybrid nucleotide sequence of no more than 1528 base pairs including a sequence defining a structural gene expressing a conjoined single strand of a multimeric TNFSF-SPD fusion protein, said structural gene having a nucleotide base sequence selected from members of the group consisting of SEQ ID NO 1, SEQ ID NO 3 and SEQ ID NO 5.
2. (Withdrawn) The DNA segment according to claim 1, wherein the structural gene comprises:
a segment expressing a single hybrid amino acid chain of TNFSF-SPD, said segment having
a first SPD nucleotide base sequence of SEQ ID NO 1, from base 32 to base 799, and
a second sequence, expressing a portion of TNFSF stalk, selected from members of the group consisting of SEQ ID NO 1, from base 800 to base 1444, SEQ ID NO 3, from base 800 to base 1528, and SEQ ID NO 5, from base 800 to base 1441.
3. (Withdrawn) A recombinant DNA molecule comprising a vector operatively linked to an exogenous DNA segment defining a structural gene expressing a single amino acid chain of TNFSF-SPD, said structural gene having
a nucleotide base sequence selected from members of the group consisting of SEQ ID NO 1, SEQ ID NO 3 and SEQ ID NO 5, any functional equivalents and modification thereof,
and
an appropriate promoter for driving the expression of said structural gene in a compatible host organism.
4. (Withdrawn) The recombinant DNA molecule as described in claim 3 wherein said host organism is E. coli.
5. (Withdrawn) The recombinant DNA molecule as described in claim 3, wherein said host organism is a yeast.

6. (Withdrawn) The recombinant DNA molecule as described in claim 3, wherein said host organism is a higher plant or animal.

7. (Currently Amended) A multimeric tumor necrosis factor super family - pulmonary surfactant protein D (TNFSF-SPD) fusion protein, comprising: a plurality of polypeptide trimers,

i) a first trimer consisting of peptide strands selected from the group consisting of members of the TNF superfamily (TNFSF) of ligands LTA, TNF, LTB, TNFSF4 - TNFSF18, and

ii) a second trimer strand from a collectin molecule (SPD) comprising a stalk region; each first TNSF trimer conjoined to the second SPD trimer strand, wherein said first trimer ligand strand is substituted for native carbohydrate recognition domains (CRD) of the collectin molecules of the second trimer strand, and wherein said conjoined TNFSF-collectin strands are covalently bound in parallel to each other within the stalk region, thereby forming a multimeric fusion protein comprising a plurality of trimeric hybrid polypeptide strands radiating from a covalently bound center hub of the molecule, the free end of each trimeric radiating strand having a TNFSF trimer moiety attached.

8. (Currently Amended) The multimeric fusion protein according to claim 7, wherein the TNFSF trimer moiety is one selected from the group consisting of ligands of the tumor necrosis factor super family LTA, TNF, LTB, and TNFSF4 to TNFSF 18 as cited by the Human Gene Nomenclature Committee, modifications thereof, and their immuno-stimulatory functional equivalents.

9. (Withdrawn) A method for preparing a CD40-SPD multimeric fusion polypeptide, comprising the steps of:

initiating a culture, in a nutrient medium, of prokaryotic or eukaryotic host cells transformed with a recombinant DNA molecule including an expression vector, appropriate for said cells, operatively linked to an exogenous DNA segment defining a structural gene for CD40-SPD ligand, said structural gene having a nucleotide base sequence of SEQ ID NO 1 from about base 32 to about base 1444; and

maintaining said culture for a time period sufficient for said cells to express said multimeric molecule.

10. (Withdrawn) A method of producing a secreted, large, biologically active, multimeric tumor necrosis factor superfamily ligand fusion protein chimera that is highly immunogenic and not readily diffusible, comprising:

introducing into a host cell a first chimeric DNA construct including a transcriptional promoter operatively linked to a first secretory signal sequence, followed downstream by, and in proper reading frame with

a first DNA sequence encoding a polypeptide chain of a first TNFSF ligand requiring multimerization for biological activity, joined to

a second DNA sequence encoding a collectin polypeptide at the site where the collectin's CRD was purposefully removed,

introducing into said host cell a second DNA construct including a transcriptional promoter operably linked to a second secretory signal sequence followed downstream by, and in proper reading frame with,

a third DNA sequence encoding a second polypeptide chain of a second TNFSF ligand, joined to

a fourth DNA sequence encoding a collectin polypeptide, wherein the collectin's CRD was purposefully removed;

growing said host cell in an appropriate growth medium under physiological conditions to allow the secretion of a large multimerized polypeptide fusion protein,

wherein said first polypeptide chain of a TNFSF-SPD protein is bound by parallel bonding of the respective collectin domain trimer to said second polypeptide chain of a different TNFSF-SPD polypeptide trimer, and wherein said multimerized polypeptide fusion protein exhibits biological activity characteristic of both membrane-attached TNFSFs; and

isolating said biologically active, multimerized TNFSF-SPD polypeptide fusion from said host cell.

11. (Withdrawn) The method according to claim 10, wherein the chimeric reactant compounds are humanized to guard against destruction by a potential human recipient's immune system.

12. (Withdrawn) A method of preparing a multimeric TNFSF-SPD ligand fusion protein, comprising:

- preparing a first DNA segment coding for a strand of an exposed extracellular portion of TNFSF;

- preparing a second DNA segment coding for a collectin polypeptide strand, wherein said collectin's CFD domain of the strand has been removed;

- conjoining said first and second DNAs in proper reading frame, therein creating a TNFSF-collectin DNA construct;

- inserting said construct into an expression vector system;

- introducing said vector system into an appropriate cell in culture under suitable conditions;

- harvesting and purifying spent medium from said culture; and

- assaying for presence of multimeric TNFSF-collectin fusion protein.

13. (Withdrawn) A method for stimulating the immune response in potentially immunocompetent cells using multimeric TNFSF fusion proteins, comprising:

- contacting said cells with said multimeric TNFSF fusion proteins, wherein said cells are induced to proliferate.

14. (Withdrawn) The method according to claim 15, wherein the cells are resting B cells.

15. (Withdrawn) A method for increasing antigenicity of cells, comprising: contacting said cells with said multimeric TNFSF fusion proteins, wherein said cells are tumor cells or HIV positive cells.

Complete Listing of all Claims (Marked-up Version)

Please amend claims 7 and 8 as set forth below:

1. (Withdrawn) A hybrid nucleotide sequence of no more than 1528 base pairs including a sequence defining a structural gene expressing a conjoined single strand of a multimeric TNFSF-SPD fusion protein, said structural gene having a nucleotide base sequence selected from members of the group consisting of SEQ ID NO 1, SEQ ID NO 3 and SEQ ID NO 5.
2. (Withdrawn) The DNA segment according to claim 1, wherein the structural gene comprises:
a segment expressing a single hybrid amino acid chain of TNFSF-SPD, said segment having
a first SPD nucleotide base sequence of SEQ ID NO 1, from base 32 to base 799, and
a second sequence, expressing a portion of TNFSF stalk, selected from members of the group consisting of SEQ ID NO 1, from base 800 to base 1444, SEQ ID NO 3, from base 800 to base 1528, and SEQ ID NO 5, from base 800 to base 1441.
3. (Withdrawn) A recombinant DNA molecule comprising a vector operatively linked to an exogenous DNA segment defining a structural gene expressing a single amino acid chain of TNFSF-SPD, said structural gene having
a nucleotide base sequence selected from members of the group consisting of SEQ ID NO 1, SEQ ID NO 3 and SEQ ID NO 5, any functional equivalents and modification thereof,
and
an appropriate promoter for driving the expression of said structural gene in a compatible host organism.
4. (Withdrawn) The recombinant DNA molecule as described in claim 3 wherein said host organism is *E. coli*.
5. (Withdrawn) The recombinant DNA molecule as described in claim 3, wherein said host organism is a yeast.

6. (Withdrawn) The recombinant DNA molecule as described in claim 3, wherein said host organism is a higher plant or animal.

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i) a first trimer consisting of peptide strands selected from the group consisting of members of the TNF superfamily (TNFSF) of ligands LTA, TNF, LTB, TNFSF4 - TNFSF18, and

ii) a second trimer strand from a collectin molecule (SPD) comprising a stalk region; each first TNSF trimer conjoined to ~~[[a]]~~ the second SPD ~~polypeptide~~ trimer strand ~~from a collectin molecule~~,

wherein said first trimer ligand strand is substituted for native carbohydrate recognition domains (CRD) of the collectin molecules of the second trimer strand, and wherein said conjoined TNFSF-collectin strands are covalently bound in parallel to each other within the stalk region, ~~therein~~ thereby forming a multimeric fusion protein comprising a plurality of trimeric hybrid polypeptide strands radiating from a covalently bound center hub of the molecule, the free end of each trimeric radiating strand having a TNFSF trimer moiety attached.

8. (Currently Amended) The multimeric fusion protein according to claim ~~[[6]]~~ 7, wherein the TNFSF trimer moiety is one selected from the group consisting of ligands of the tumor necrosis factor super family LTA, TNF, LTB, and TNFSF4 to TNFSF 18 ~~as in Table II as cited by the~~ Human Gene Nomenclature Committee, modifications thereof, and their immuno-stimulatory functional equivalents, ~~and modifications thereof~~.

9. (Withdrawn) A method for preparing a CD40-SPD multimeric fusion polypeptide, comprising the steps of:

initiating a culture, in a nutrient medium, of prokaryotic or eukaryotic host cells transformed with a recombinant DNA molecule including an expression vector, appropriate for said cells, operatively linked to an exogenous DNA segment defining a structural gene for CD40-SPD ligand,

said structural gene having a nucleotide base sequence of SEQ ID NO 1 from about base 32 to about base 1444; and

maintaining said culture for a time period sufficient for said cells to express said multimeric molecule.

10. (Withdrawn) A method of producing a secreted, large, biologically active, multimeric tumor necrosis factor superfamily ligand fusion protein chimera that is highly immunogenic and not readily diffusible, comprising:

introducing into a host cell a first chimeric DNA construct including a transcriptional promoter operatively linked to a first secretory signal sequence, followed downstream by, and in proper reading frame with

a first DNA sequence encoding a polypeptide chain of a first TNFSF ligand requiring multimerization for biological activity, joined to

a second DNA sequence encoding a collectin polypeptide at the site where the collectin's CRD was purposefully removed,

introducing into said host cell a second DNA construct including a transcriptional promoter operably linked to a second secretory signal sequence followed downstream by, and in proper reading frame with,

a third DNA sequence encoding a second polypeptide chain of a second TNFSF ligand, joined to

a fourth DNA sequence encoding a collectin polypeptide, wherein the collectin's CRD was purposefully removed;

growing said host cell in an appropriate growth medium under physiological conditions to allow the secretion of a large multimerized polypeptide fusion protein,

wherein said first polypeptide chain of a TNFSF-SPD protein is bound by parallel bonding of the respective collectin domain trimer to said second polypeptide chain of a different TNFSF-SPD polypeptide trimer, and wherein said multimerized polypeptide fusion protein exhibits biological activity characteristic of both membrane-attached TNFSFs; and

isolating said biologically active, multimerized TNFSF-SPD polypeptide fusion from said host cell.

11. (Withdrawn) The method according to claim 10, wherein the chimeric reactant compounds are humanized to guard against destruction by a potential human recipient's immune system.

12. (Withdrawn) A method of preparing a multimeric TNFSF-SPD ligand fusion protein, comprising:

- preparing a first DNA segment coding for a strand of an exposed extracellular portion of TNFSF;

- preparing a second DNA segment coding for a collectin polypeptide strand, wherein said collectin's CFD domain of the strand has been removed;

- conjoining said first and second DNAs in proper reading frame, therein creating a TNFSF-collectin DNA construct;

- inserting said construct into an expression vector system;

- introducing said vector system into an appropriate cell in culture under suitable conditions;

- harvesting and purifying spent medium from said culture; and

- assaying for presence of multimeric TNFSF-collectin fusion protein.

13. (Withdrawn) A method for stimulating the immune response in potentially immunocompetent cells using multimeric TNFSF fusion proteins, comprising:

- contacting said cells with said multimeric TNFSF fusion proteins, wherein said cells are induced to proliferate.

14. (Withdrawn) The method according to claim 15, wherein the cells are resting B cells.

15. (Withdrawn) A method for increasing antigenicity of cells, comprising: contacting said cells with said multimeric TNFSF fusion proteins, wherein said cells are tumor cells or HIV positive cells.